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# The Influence of the time and Temperature Binomium in the Process of Extraction of Bioactive Compounds from Guaraná (Paullinia cupana)

# A Influência do Binômio Tempo e Temperatura no Processo de Extração de Compostos Bioativos do Guaraná (Paullinia cupana)

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***Keywords*— HPLC, Spray dryer, Maltodextrin, Yield.**
***Palavras-chave*— HPLC, Spray drying, Maltodextrina, Rendimento.**

***Abstract*** *— Guarana is a plant native to the state of Amazonas that found favorable soil and climate conditions for its development in southern Bahia. It is recognized worldwide for its medicinal and stimulant properties from the presence of alkaloids from the methylxanthine group, such as caffeine. The caffeine content in guarana can reach 6%, a content four times higher than arabica coffee, which makes it a useful raw material for the caffeine extraction process. Among the caffeine extraction processes, there is the method of extraction by chemical solvents, which, in addition to being expensive, are very harmful to the environment, so an alternative is the use of water as a solvent, which also has a good affinity in the process. extraction and does not harm the environment like chemical solvents. The objective of this project is to optimize the process of extracting caffeine from guarana seeds, using water as a solvent, through an experimental design. Chromatographic and microbiological analyzes will be carried out and the concentration of the extract will be performed with the highest yield by the spray drying method. Data evaluation will be performed using the Statistica software version 10, for analysis of variance with a 95% confidence interval. It is expected that, with this project, extracts rich in caffeine will be obtained and that it will be able to determine which variables influenced the caffeine extraction process, so that in this way they can be worked more deeply. Keywords: HPLC, Spray drying, Maltodextrin, Yield.*

***Resumo*** *— O guaraná é uma planta nativa do estado do Amazonas que encontrou condições edafoclimáticas favoráveis para o seu*

*desenvolvimento no sul da Bahia. É reconhecida mundialmente pelas suas propriedades medicinais e estimulantes proveniente da presença de alcaloides do grupo das metilxantinas, como a cafeína. O teor de cafeína no guaraná pode chegar a 6%, teor quatro vezes maior que o café arábica, que, o torna uma matéria prima útil para o processo de extração de cafeína. Dentre os processos de extração de cafeína, tem se o método de extração por solventes químicos, que, além de caro, agridem muito o meio ambiente, assim, uma alternativa é a utilização da água como solvente, que também possui uma boa afinidade no processo de extração e não agride o meio ambiente como os solventes químicos. O objetivo deste projeto é otimizar o processo de extração de cafeína de sementes de guaraná, utilizando a água como solvente, por meio de um planejamento experimental. Será realizada análises cromatográficas, microbiológicas e a concentração do extrato com maior rendimento pelo método de secagem em Spray drying. A avaliação dos dados será realizada utilizando o software Statistica versão 10, para análise de variância com intervalo de confiança de 95%. Espera se que, com o presente projeto obtenha extratos ricos em cafeína e que consiga determinar quais foram as variáveis que influenciaram no processo de extração de cafeína, para que dessa forma sejam trabalhas mais profundamente.*

## I. INTRODUCTION

Guarana (Paullinia cupana) is a plant native to the state of Amazonas that is recognized worldwide for its medicinal and stimulant properties.This stimulant power comes from the presence of methyl xanthine-type alkaloids, with a greater predominance of caffeine (SANTOS, 2017).

Although guaraná is of Amazonian origin, this species found favorable soil and climate conditions for its development in southern Bahia due to rainfall, temperature, relative humidity and the production system adopted by the state (SANTOS, 2017).According to Conab (2019), Bahia was the state to conquer the first place in the guaraná production ranking in 2018, Amazonas occupies the second position, followed by Mato Grosso.

The guarana seed can present a percentage of approximately 6% of caffeine, a content four times higher than coffee, which shows its stimulant power (NOGUEIRA, 2017).

In addition to caffeine, the chemical composition of guarana is characterized by the presence of theophylline, theobromine, tannins, gallic acid, saponins, catechins, epicatechins, flavonoids, phenolic compounds, antioxidants, among other compounds in lower concentrations (PINTO, 2012).

For the pharmaceutical, food and cosmetic industries, caffeine is of extreme value, because, when consumed in a moderate way, this alkaloid acts in an antagonistic way to adenosine receptors, which provides an increase in the individual's state of alertness, promoting aimprovement in the association of ideas and intellectual activities, greater resistance to fatigue and increased well-being (SANTOS, 2017).

In the literature, it is possible to find several studies on caffeine extraction and concentration (NOGUEIRA, 2017).Among the methods employed are the use of organic solvents with the affinity of caffeine, which aims to extract this alkaloid.The solvents used are: 2 dichloromethane, ethyl acetate, benzene, chloroform, ether, alcohol, trichlorethylene, carbon tetrachloride, acetone, among others (SANTO, 2016).

However, for the industry, the use of these solvents results in a high value in the production line, because, in addition to having a high price and having the need to remove the solvent through distillation, these solvents harm the environment. (SANTO, 2016).

An alternative is the use of water in the caffeine extraction process, which also has a good affinity, has low value and does not harm the environment when compared to chemical solvents (LEITE, 2009).

During the caffeine extraction process, attention must be paid to several factors such as: extraction time and temperature, as these directly influence the final result. Thus, the study of these factors is necessary, so that it becomes possible to understand the system and the interactions between them, so that maximum yields are obtained during the extraction process (SANTO, 2016).

The present work aims to obtain and characterize extracts of guarana (Paullinia cupana) in powder form subjected to different temperatures and extraction times.

## II. MATERIALS AND METHODS

2.1 Plant material

The organic guarana seeds were supplied by Asscorp Alimentos LTDA acquired from the Onça project in the municipality of Taperoá, BA, a city located in the Territory of Citizenship of Baixo Sul da Bahia, from the 2019 harvest.original, stored in transparent polyethylene bags and packed in cardboard boxes.The guarana beans were sent to the company Moinhos Vieira, located in Rio de Janeiro, where they were ground to a particle size of 0.5 µm.The powder was stored in transparent polyethylene bags and packed in cardboard boxes and sent to the Instituto Federal do Norte de Minas Gerais-Salinascampus.

2.2 Preparation of extracts

Factorial experimental design

A 22 factorial design was adopted with triplicate at the central point.The central point was necessary for the calculation of the experimental error. In the factorial design, the influence of Time (72 and 12 hours) and Temperature (60 and 80 °C) was evaluated and at the central point, the time of 48 hours and the temperature of 70 °C were evaluated, as shown in Table 1The 22-factor design assays at different levels were performed in duplicates.Table 2 shows the tests performed in the present study.The sample weight of 50 g, stirring speed of 220 rpm, volume of 100 mL of water and granulometry of 0.5 µm were kept fixed.

*Table 1: Actual values assigned to experimental design variables*

| Levels | Temperature (ºC) | Time(hours) |
|---|---|---|
| 1 | 60 | 12 |
| -1 | 80 | 72 |
| PC | 70 | 48 |

Source: From the Author.

*Table 2: Experimental Design 22 with triplicate at the central point.*

| Treatment | Temperature (ºC) | Time (hours) |
|---|---|---|
| 1 | 80 | 72 |
| 2 | 80 | 72 |
| 3 | 80 | 12 |
| 4 | 80 | 12 |
| 5 | 60 | 12 |
| 6 | 60 | 12 |
| 7 | 60 | 72 |
| 8 | 60 | 72 |
| 9 | 70 | 48 |
| 10 | 70 | 48 |
| 11 | 70 | 48 |

Source: From the Author.

2.3 Preparation of extracts

The preparation of aqueous extracts was carried out according to an experimental design as shown in Table 2. Sterile distilled water was used, autoclaved at 121 °C for 15 minutes.

50 grams of guarana powder were weighed on a Martes analytical balance, model BL 3200H and transferred to erlenmeyers and then 100 mL of distilled water was added and homogenization was carried out. The erlenmeyers were sealed with aluminum foil and masking tape and placed in a Nova Técnica brand water bath, model NT 232 Dubnoff, under agitation at 220 rpm. After that, a first filtration of the extract was carried out in Voil Fabric, then a second filtration was carried out, through the vacuum method.

After that, the extracts were transferred to falcon tubes and centrifuged (Centrifuga Mad Lab CT-5000) at 3000 rpm for 3 minutes. The extracts were stored in amber glass and the extract residues were dried in a conventional oven at a temperature of 55 to 60 °C until constant weight and stored in laminated Zip Lock bags. Both were taken to the BOD oven at 10 °C until the time of analysis.

2.4 Physicochemical analysis of guarana powder

The moisture content was determined by gravimetry in a conventional oven at 105 °C, until constant mass, according to Instituto Adolfo Lutz (2008).The determination of ash (fixed mineral residue) was performed by gravimetric method from the incineration at 550 ºC in the muffle for an average of 6 hours according to the Instituto Adolfo Lutz (2008).Crude protein determination was performed using the Kjeldahl method, according to Instituto Adolfo Lutz (2008).The lipid content was determined by the Soxhlet method, according to Instituto Adolfo Lutz (2008).All physicochemical analyzes were performed in triplicate.

Sample Preparation

For the preparation of the samples, an aliquot of 0.5 mL of the guarana extracts was removed and for the guarana powder and the residue of the extracts, 0.02 g of sample was used.5 mL of extracting solution (85% H20 Milli-Q,

acidified with 0.3% acetic acid, and 15% Methanol) was added to falcon centrifuge tubes containing the samples.The tubes were taken to an ultrasonic bath (SoniClean 6) under agitation for 15 minutes, followed by a water bath at 60 °C for 10 minutes, and then centrifuged (3000 rpm for 15 minutes) for better separation.These extracts were used for the analysis of Caffeine, Theobromine, Catechin, Epicatechin Flavonoids, Phenolic Compounds and Antioxidants.

Total Phenolic Compounds (CFT)

For the determination of total phenolic compounds, the methodology used by Lee et al. (2003). An aliquot of 0.5 mL of sample was added to a test tube and 2.5 mL of 10% Folin Ciocalteau and 2.0 mL of 7.5% Sodium Carbonate solution were added, totaling 5.0 mL corresponding to each trial. The obtained solution was taken to a water bath at 40 °C for 15 minutes. After that, the samples were left to rest for 30 minutes under the protection of light. The reading was performed in a spectrophotometer (UV VIS SCAN model 0898UV2) in a quartz cuvette, at an absorbance of 750 nm. The blank was made by adding extractor solution in place of the sample. The analysis was performed in duplicate.

The calibration curve was obtained from the aqueous solution of Gallic Acid at concentrations between 10 and 100 µg AG.mL-1. The CFT content results were expressed as Gallic Acid equivalents (g AG.100g-1) and calculated using a curve constructed with concentrations of the standard. The R2 obtained was 0.993.

Total Flavonoids (FT)

The total flavonoid content was adapted by the method proposed by Zhishen, Mengcheng; Janming (1999). An aliquot of 0.5 mL of the extract, 2.5 mL of distilled water and 0.15 mL of 5% sodium nitrite (NaNO2) were added to test tubes, vortexed and left to rest for 6 minutes. Given the time, 0.3 mL of 10% aluminum chloride (AICI3) was added, stirred and left to stand for 5 minutes. After that, 1 mL of 1M sodium hydroxide (NaOH) was added and the remainder of water was added to 5 mL. The spectrophotometer reading (UV VIS SCAN model 0898UV2) in a quartz cuvette, at absorbance of 510 nm. The blank was performed by adding methanol in place of the sample. The analysis was performed in duplicate.

The calibration curve was obtained from the catechin solution at concentrations of 20 and 200 µg Ca.mL-1. Total flavonoid content results were expressed as Catechin equivalents (g Ca.100g-1). The R2 obtained was 0.976.

Antioxidant Analysis

The total antioxidant activity was determined using the DPPH method according to the methodology used by Brand-Williams;Cuvelier;Berset (1995), which consists in the capture of the DPPH radical (2,2-diphenyl-1-picryl-hydrazyl) by antioxidants contained in the sample, using the DPPH standard solution.

0,1 mL of sample and 3.9 mL of DPPH were added to test tubes, the solution was homogenized and after standing for 30 minutes under the protection of light, the reading was carried out in a quartz cuvette, using the spectrophotometer.(UV VIS SCAN model 0898UV2) with absorbance of 515 nm.The blank was performed with ethyl alcohol in place of the sample.The results for the DPPH assay were expressed in percentage of inhibition (%I) of DPPH present in the extracts capable of decreasing the initial concentration of DPPH in 30 minutes.The percentage of inhibition of the DPPH radical was calculated according to equation 1:

(Equation 1) DPPH %E=Control - Sample sample x 100

Where:

Ac= DPPH absorbance

Aa= Sample absorbance

Quantification of Caffeine, Theobromine, Catechin and Epicatechin in High Performance Liquid Chromatography (HPLC)

The high performance liquid chromatography method was used to quantify caffeine and theobromine and major phenolic compounds in guarana samples ((+)-catechin and (-)-epicatechin), this method was based on the methodology used by Risner, (2008). ) and Gonçalves (2016).

Samples were filtered with sterile 0.22 µm filters and stored in eppendorf until analysis.

A SHIMADZU DGU-20A5R High Performance Liquid Chromatograph was used, equipped with a manual injector, with an injection volume of 20 µm, a Shim-pack column PREP-ODS(H)KIT (H) (octadecylsilane) 250 mm x 4.6 mm and with an ultraviolet wavelength of 280nm at a flow rate of 0.5 mL/min.

The mobile phase composite gradient was established as: A (Methanol) and B (H2O Milli-Q with 0.3% Acetic Acid) with a total run time of 28 minutes and a temperature of 30 °C ± 2.

2.5 Statistical Analysis

The results obtained in the present study were statistically evaluated using the Analysis of Variance (ANOVA) methods, followed by a Tukey mean comparison test and Contrast Analysis.For this, the SISVAR 5.7 software was used.All statistical tests used had a 5% significance level ($p \leq 0.05$).

## III. RESULTS AND DISCUSSIONS

Physicochemical characterization of guarana poder

The results obtained from the physical-chemical characterization of guarana powder are shown in Table 3, below. This type of analysis mainly aims to analyze the quality of the raw material, as well as quantify the active principles present in it. According to Pellozo (2005), the high presence of humidity is a factor that can not only favor microbial contamination but also triggers enzymatic reactions culminating in the degradation of active principles. As for the ash content, it is an indication of the possibility of containing inorganic impurities in guarana powder.

The moisture and ash content of guarana powder was approximately 8.7% and 2.3%, respectively. According to the Ministry of Agriculture, Livestock and Supply (BRASIL, 1982), the moisture content should be a maximum of 12% and 2% ash. The National Commission of Norms and Standards for Food (CNNPA) of the Ministry of Health (BRASIL, 1978), says that the moisture content should be a maximum of 7% and the ash content 2%. However, the Brazilian Pharmacopoeia (2003) determines that the ash content should be a maximum of 3%.

As the guarana powder used in this experiment presented a moisture content below 12%, it is within the limits established by MAPA (BRASIL, 1982), however, it presented a higher content for ash.

As for the protein and lipid content, the values obtained were approximately 8.4% and 4.6%, respectively. These results were lower than those by Nazaré (1998), who analyzed guarana powder and obtained 13.69% of proteins and 5.57% of lipids. Martins (2010) also obtained a higher protein content (12.84%) in his study, however, the lipid content was lower (2.85%) than that obtained in the present research.

The stimulant and prolonged effect of guarana is attributed to the presence of a complex formed between methylxanthines and condensed tannins (PELLOZO, 2005). Pellozo (2005) also states that the total tannin content is attributed to the presence of substances that are composed of interconnected monomeric units of proanthocyanidins and/or prototannins (catechin and epicatechin).

The caffeine and theobromine content obtained was 3.902 g.100g-1 and 1.627 g.100g-1, respectively.The caffeine content was close to that found by Souza (2010) and Pereira (2011), but the theobromine content in this study was higher.However, the caffeine content was lower than that found by Nazaré (1998), who obtained a caffeine content of 4.45% when analyzing the guarana powder sample.

*Table 3: Physicochemical and chemical characterization of Guarana poder.*

| Determinations | Results |
|---|---|
| Humidity (%) | 8,709 ± 0,291 |
| Lipids (%) | 4.462 ± 0.342 |
| Protein (%) | 8.432 ± 0.396 |
| Gray (%) | 2.274 ± 0.013 |
| Caffeine (g.100g-1) | 3.902 ± 0.186 |
| Theobromine (g.100g-1) | 1.627 ± 0.114 |
| Epicatechin (g.100g-1) | 2.008 ± 0.102 |
| Catechin (g.100g-1) | 0.146 ± 0.57 |
| FT (gCa*.100g-1) | 2.030 ± 0.347 |
| CFT (gAG**.100g-1) | 17.797 ± 1.751 |
| %I DPPH *** | 60,879 ± 2,542 |

Results of means ± standard deviation.

*Catechin;** Gallic Acid;*** Inhibition Percentage.

The content of epicatechin and catechin found in guarana powder was 2.008g.100g-1 and 0.146 g.100g-1, respectively.These values are lower than those found by Souza (2010), who obtained a catechin and epicatechin value of 0.87 g.100g-1 and 0.61 g.100g-1, respectively.

According to Gomes (2018), the antioxidant activity of guarana is associated with phenolic compounds such as tannins.Phenolic compounds are responsible for color, astringency, aroma, oxidative stability and are included in the category of free radical scavengers.These compounds are distributed in substances such as phenolic acids, flavonoids, among others.

The content of phenolic compounds was given as a percentage of %galic acid, the value obtained (Table 4) in the present study was 17.797 gAG.100g-1, about 178 mg AG.g-1, a value higher than that found by Pereira (2011), who obtained a content of 128.64 mg AG.g-1 and lower than the research carried out by Gomes (2018) who analyzed different batches of guarana powder, obtained an average content of 440 mg.AG.g-1 and 270 mg.AG.g1.

Flavonoids are considered a secondary group of the class of low molecular weight phenolic compounds, they can be presented as flavonols, flavones, flavanones, catechins, anthocyanins, isoflavones and chalcones. These compounds also have a direct action in the scavenging of free radicals, therefore presenting antioxidant activity.

In the present study, the TF content was given in catechin (g Ca). In this way, guarana powder showed about 20.30 mg Ca per gram of sample. In the work carried out by Gomes (2018), the flavonoid content was given in amount of catechol (CAT), the analyzed batches showed an average amount of 288.75 mg CAT.g-1 and 150.09 mg CAT.g-1.

As for the antioxidant activity of guarana powder, it was expressed in terms of DPPH Radical Inhibition Percentage (%I). The value obtained was approximately 61%I DPPH, a value below those obtained by Gomes (2018), who obtained averages of 79%I DPPH and 70%I DPPH.

Gomes (2018) states that the high antioxidant activity of guarana powder is mainly due to the amount of phenolic compounds present. Because of this, guarana powder has been the focus of some research, as it is a natural product and protects the body from damage caused by free radicals, preventing various diseases.

Experimental Design 22 with triplicate at the central point The statistical analysis of the data consisted of two stages. In the first stage, the statistics of the means obtained from the factorial design 22 were performed, that is, the first 8 trials were analyzed. Tukey's Test and Analysis of Variance (ANOVA) were applied to these data. In the second stage, the 11 factorial design trials were analyzed. In this step, in addition to the Analysis of Variance (ANOVA) and the Tukey Test, the Contrast Test was applied, which aims to evaluate the general average obtained by the 22 factorial design in relation to the central point (additional treatment). In both stages, the tests were performed with a significance level of 5% ($p \leq 0.05$).

The significance of the effects of temperature, time and the interaction between them for planning 22 was determined by analysis of variance, where, for each response of the significance of the effects, it was verified using the p values. P values less than or equal to 0.05 indicate that the variable is significant in the experimental domain studied, with a confidence level of 95%. Table 4 shows the mean square values obtained by the analysis of variance, it also shows the source of variation (FV) and degrees of freedom (GL) for the analysis of caffeine, theobromine, catechin and epicatechin in guarana extracts.

*Table 4: Analysis of variance of the results obtained from the 22 factorial design *Significant; **Not significant. Significance level of 5%*

| FV | GL | Caffeine | Theobromine | Catechin | Epicatechin |
|---|---|---|---|---|---|
| Temperature (°C) | 1 | 0,148513s* | 0,044253s | 0,000761s | 0,003321ns |
| Time (hours) | 1 | 0,000545ns** | 0,009870ns | 0,000008ns | 0,000006ns |
| Temperature*Time | 1 | 0,000365ns | 0,018528ns | 0,000450ns | 0,002556ns |
| Error | 4 | 0,001531 | 0,002376 | 0,000074 | 0,000567 |
| Total corrected | 7 | | | | |

Through analysis of variance, it was observed that temperature was significant at a significance level of 5% ($p \leq 0.05$) for caffeine, theobromine and catechin, and not significant for epicatechin. Time was not significant for the compounds analyzed in Table 4. The same occurred when the interaction between temperature and time was performed.

In a study carried out by Paredes et al. (2016), the process of extracting caffeine from guarana seeds was evaluated, showing that the temperature had a greater influence on the extractive process when compared to the time used. The author also states that, in addition to these factors, the granulometry and the type of solvent influence the extraction process, since, in addition to caffeine, guarana has other substances such as: theophylline, theobromine, alkaloids, terpenes, tannins, flavonoids, saponins. , pigments, it is good to extract caffeine, and that studies of these factors are necessary, so that it is possible to understand the system and interactions to obtain maximum yields.

Table 4.1 shows the mean square values obtained by the analysis of variance, it also shows the source of variation (FV) and degrees of freedom (GL) in the analysis of flavonoids, phenolic compounds and %I DPPH of guarana extracts.

*Table 4.1: Analysis of variance of the results obtained from the factorial design 22*

| FV | GL | %I DPPH | Flavonoids | Phenolic Compounds |
|---|---|---|---|---|
| Temperature (°C) | 1 | 21,014645s* | 0,026796s | 0,138601ns |
| Time (hours) | 1 | 42,007778s | 0,003081s | 0,029161ns |
| Temperature*Time | 1 | 47,638561s | 0,00023ns** | 0,014028ns |
| Error | 4 | 1,496649 | 0,000353 | 0,090844 |
| Total corrected | 7 | | | |

Significant;**Not significant.Significance level of 5% (p≤0.05).

When evaluating the I DPPH, temperature, time and the interaction between them, it proved to be significant. In relation to flavonoids, both time and temperature proved to be significant, however, it was not significant when the interaction between the two was effected. As for phenolic compounds, there was no significant difference in relation to the sources of variations analyzed. All data contained in Table 4.1 were evaluated at a significance level of 5% (p≤0.05).

In a research carried out by Schafranski et al. (2019), on extractions of bioactive compounds and antioxidant activity of black mulberry leaf extracts, demonstrated that both temperature and time significantly influenced the extraction of total phenolic content. In these extracts, flavonoids, flavonoids and ortho-diphenolics were also evaluated, as well as the antioxidant activity against the DPPH radical. A research carried out by Piovesan (2016), which evaluated extractive processes of bioactive compounds in blueberries, found that time and temperature were significant for the extraction of total phenolic compounds, while for flavonoids only the temperature was significant. And for DPPH, temperature and time were not significant.

When analyzing the means as a function of temperature, using the Tukey test (Table 5), it is noted that there was a significant difference at a significance level of 5% (p≤0.05) for caffeine, theobromine and catechin, in the however, there was no significant difference for epicatechin when the means obtained at 60 °C and 80 °C were evaluated. On the other hand, when the averages were evaluated as a function of time, all the analyzed compounds did not show a significant difference between the averages obtained at the time of 12 and 72 hours.

For the averages obtained as a function of temperature (Table 5) for %I DPPH and flavonoids, there was a significant difference between the samples at 60 °C and 80 °C at a significance level of 5% (p≤0.05), already in In relation to phenolic compounds, there was no difference between the means.

When the means were analyzed in relation to time (Table 5.1), it was observed that there was a difference between the means of flavonoids and % IDPPH at a significance level of 5% (p≤0.05), while phenolic compounds did not differ between yes.

*Table 5: Tukey's test of means as a function of time and temperature*

| Variable | Treatment | Caffeine (g.100g-1) | Theobromine (g.100g-1) | Catechin (g.100g-1) | Epicatechin (g.100g-1) |
|---|---|---|---|---|---|
| Temperature (°C) | 60 | 0,688±0,041a | 0,215±0,109a | 0,045±0,010a | 0,116±0,034a |
| | 80 | 0,416±0,025b | 0,066±0,024b | 0,025±0,011b | 0,075±0,020a |
| Time (hours) | 12 | 0,560±0,168a | 0,176±0,146a | 0,037±0,005a | 0,095±0,010a |
| | 72 | 0,544±0,152a | 0,105±0,052a | 0,033±0,021a | 0,096±0,051a |

Values obtained from means ± standard deviation.Values that have the same letters in the same column are similar at a significance level of p≤0.05, and those that have different letters are different from each other, according to Tukey's test.

*Table 5.1: Tukey test result as a function of time and temperature Values obtained from means ± standard deviation.Values that have the same letters in the same column are similar at a significance level of p≤0.05, and those that have different letters are different from each other, according to Tukey's test.*

| Variable | Temperature | %I DPPH | Flavonoids (gCa.100g-1) | Phenolic Compounds (gAG.100g-1) |
|---|---|---|---|---|
| Temperature (°C) | 60 | 88,897±0,182a | 0,226±0,035a | 1,271±0,0,346a |
| | 80 | 85,655±5,643b | 0,110±0,017b | 1,007±0,124a |
| Time (hours) | 12 | 89,568±0,965a | 0,188±0,073a | 1,199±0,110a |
| | 72 | 84,985±4,893b | 0,149±0,063b | 1,079±0,399a |

In the analysis of variance for the treatments used during the extraction process, it was shown to be significant for caffeine, theobromine, % IDPPH and flavonoids and not significant for catechin, epicatechin and phenolic compounds (Tables 6 and 6.1) at a significance level of 5%.(p≤0.05).

*Table 6: Result of analysis of variance for 22 factorial design with triplicate at the central point.*

| FV | GL | Caffeine (g.100g-1) | Theobromine (g.100g-1) | Catechin (g.100g-1) | Epicatechin (g.100g-1) |
|---|---|---|---|---|---|
| Treatment | 4 | 0,046211s | 0,018749s | 0,010875ns | 0,003761ns |
| Error | 6 | 0,003775 | 0,002397 | 0,008578 | 0,000940 |
| Total corrected | 10 | | | | |

s = significant;ns= not significant

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